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Urea Stimulation of Pituitary Pars Intermedia Cells of Suckling Mice under Copious Drinking

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ABSTRACT—Ultrastructural alterations in cells of the pars intermedia (PI) of the pituitary gland were investigated morphometrically in suckling mice being fed with the pelleted food (Group I), in suckling dams given a liquid diet containing 11.5% milk powder (Group II) and in those received a 11.5% liquid milk diet supplemented with 1% urea (Group III) for 5 days from day 13 to day 17 of lactation. The percent area of the cytoplasm occupied by the rough endoplasmic reticulum increased from 11.9±1.0% (Group I) through 17.9±1.0% (Group II) to 36.9±1.1% (Group III). The numerical density of immature Golgi granules also increased from 0.9±0.06 (Group I) through 1.2±0.09 (Group II) to 1.5±0.09 (Group III). Whereas the numerical density of secretory granules decreased from 2.3±0.11 (Group I) through 1.9±0.07 (Group II) to 1.0±0.05 (Group III). These results indicated that the release of secretory granules and the protein synthesis increased significantly in PI cells of suckling mice fed a liquid milk diet and, further, the urea supplement to the liquid milk diet elicited the secretion activity of PI cells more significantly than those of animals received the liquid milk diet only.

INTRODUCTION

Our previous electron microscopic and morphometric studies have demonstrated marked hypersecretion in cells of the pars intermedia (PI) of the mouse pituitary gland in response to dietary sodium restriction [1–3] suggesting a new role for the PI hormone(s) as a major pituitary factor in the regulation of aldosterone secretion by the adrenals [4, 5]. This new proposition has now been supported and confirmed by in vitro studies indicating that pro-opiomelanocortin-derived peptides such as alpha-MSH, beta-MSH and gamma-MSH are involved in the control of aldosterone secretion by the adrenals from rats and human beings with idiopathic hyperaldosteronism [6, see also references]. However, several lines of evidence have shown that the renin-angiotensin system is a prime regulator of aldosterone secretion [7, 8]. Thus, the physiological interrelationship between the pro-opiomelanocortin-derived PI prptides and the renin-angiotensin system in the regulation of alsosterone secretion has not yet been settled.

Our recent morphometric studies have also demonstrated that experimental copious drinking has an effect to cause marked hypersecretion in PI cells of the mouse pituitary [9] and that the PI cells of new-born mice indicate structural hypersecretion when, after 5 hr of separation, pups are reunited with their mother and allowed to suck their mother's breast for 1 hr [10]. These results suggest another new role of PI peptides in the regulation of hydro-mineral metabolism in mice.

On the other hand, one of the interesting behaviours of mice observed during lactation is a kind of coprophagy [11]. The suckling mice used to lick pups' urine and stools off their rump during nursing. In addition, there is a parallelism between a high blood urea concentration and the enlarged pars intermedia in mice with hereditary nephrogenic diabets inspidus [12, 13]. Then the present study was designed to investigate the effects of experimental polydipsia [9] and of combined urea and polydipsia on pituitary PI cells of suckling dams whose water consumption is about 4 times as much as that of age matched nulliparous mice. Plasma osmolality and hematocrit were also evaluated since the specificity of the effect of osmolality on aldosterone secretion has been re-

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cently reported [14, 15].

MATERIALS AND METHODS

Albino mice of the ICR/Jcl strain were housed at a temperature of 22±1°C and 12 hr-periods of light and darkness (7.00–19.00 light on). Primigravid mice were isolated single in a cage provided with a pelleted mouse food and tap water. One day after the parturition, the number of siblings was reduced and adjusted to 6 pups. On day 12 of lactation, suckling dams were divided into three groups, and the following experiments were undertaken during the period from day 13 to day 17 of lactation. The first group served as controls receiving a pelleted food and distilled water. The second group was subjected to copious drinking; animals were deprived of a pellted food and water and , instead, were fed with a liquid diet consisting of powdered milk (Yuki-zirushi Neomilk, Yukizirushi, Tokyo) at the concentration of 11.5%, the same concentration used in nursing a human baby. The third group also received a liquid diet of 11.5% powdered milk supplemented with 1% urea. The final concentration of urea used was determined in a way to keep the consumption of a urea plus milk liquid diet on a level with that of a milk liquid diet. Oral intake of a liquid diet was measured daily and the body weight of both suckling dams and pups was weighed. On day 17 of lactation, the suckling mice were killed by decapitation and the blood was collected from trunk vessels into heparinized capillary tubes. The hematocrit value was obtained after centrifugation of the blood samples for 10 min at a speed of 9,500 rpm. Plasma osmolality (mOsm/kg) was measured using Shimazu OSM-1 osmometer. The pituitaries were removed immediately after the sacrifice and small pieces of parasagittal slices of specimens were fixed in the solution of 1% glutaraldehyde and 1% paraformaldehyde with phosphate fuffer at pH 7.4 for 1 hr followed by 1% OsO_4 (pH 7.4) for another 1 hr. After dehydration specimens were embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an H-11E Hitachi Electron Microscope. Electron micrographs were taken at original magnification of 2,500 and enlarged optically at 10,000 times.

In each group approximately 50 PI cells of electron micrographs were chosen at random for morphometric analysis. The area of the cytoplasm, nucleus and Golgi region was measured

TABLE 2. Hematocrit and osmolality of suckling mice given a pelleted food and distilled water (Group I), a 11.5% milk diet (Group II) and a 11.5% milk diet added with 1% urea (Group III) on day 17 of lactation

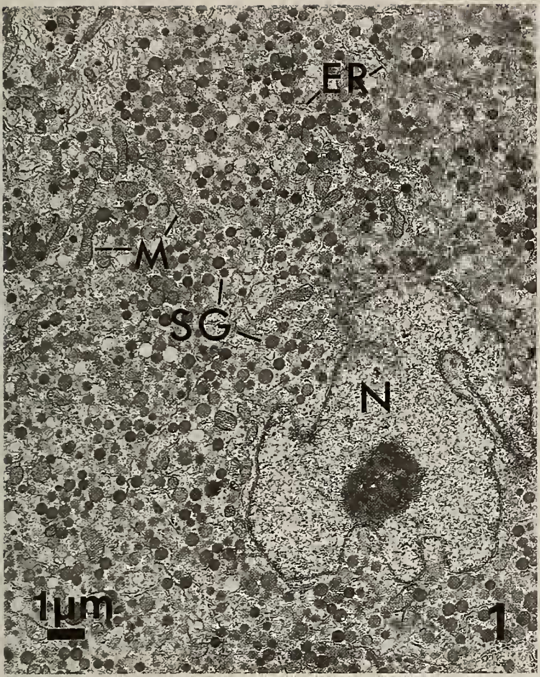
	Hematocrit (%)	Osmolality (mOsm/kg)
Pellet+D.W. (Group I)	48.7±0.6[a]	305±2.2 (3)
Milk diet (Group II)	57.4±0.6*	309±2.2 (6)
Milk+Urea (Group III)	56.5±0.5*	308±1.3 (8)

[a] Mean±S.E.M., * Group I vs. Group II and Group III, $p<0.01$. No. of animals is shown in parentheses.

TABLE 1. Water (D.W.) and liquid diet consumption (ml/10 g b.w./day) by suckling mice being fed with a pelleted food (Group I) , with a 11.5% milk diet (Group II) and with a 11.5% milk diet added with 1% urea (Group III) from day 13 to day 17 of lactation

Day of lactation	13	14	15	16	17
Pellet+D.W. (Group I)	7.5±0.8[a]	7.8±0.7	7.5±0.5	6.4±0.3	8.5±0.6 (3)
Milk diet (Group II)	13.6±0.9*	13.8±1.0*	14.3±0.7*	17.0±1.5*	16.4±1.2* (6)
Milk+Urea (Group III)	12.8±0.6*	14.0±0.7*	16.6±1.0*	17.0±1.1*	18.4±1.3* (8)

[a] Mean±S.E.M., * Group I v.s Group II and Group III, $p<0.01$ No. of animals is shown in parentheses.



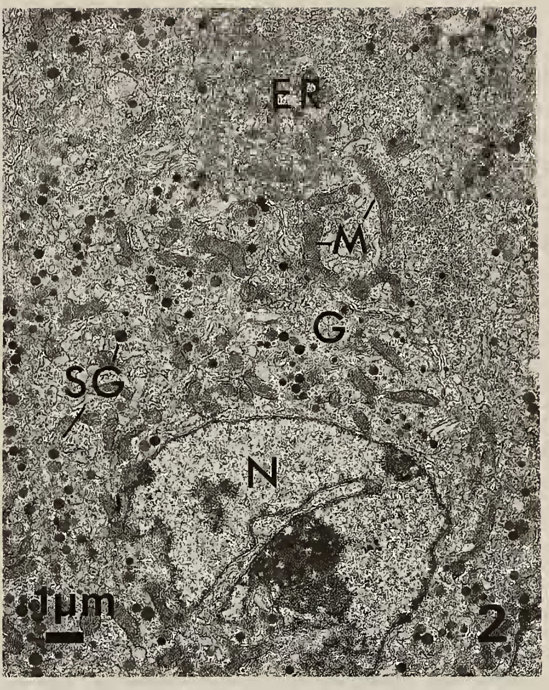


FIG. 1. Part of pituitary pars intermedia cells of the suckling mouse received pelleted food and distilled water. Note numerous secretory granules (SG), scanty rough endoplasmic reticulum (ER) and rod shaped mitochondria (M) in the cytoplasm. The nucleus (N) shows marked indentation with a prominent nucleolus.

FIG. 2. Part of pipuitary pars intermedia cells of the suckling dam fed a liquid diet containing 11.5% milk powder and 1% urea. Note well developed rough endoplasmic reticulum (ER), extensive Golgi apparatus (G), sparse secretory granules (SG) and scattering mitochondria (M). The nucleus (N) is irregular in shape.

TABLE 3. The percent area of the rough endoplastic reticulum (% r-ER), the numerical density of secretory granules (Secretory G./μm^2) and of immature Golgi granules (Golgi G./μm^2) in the Golgi region, and the total number of Golgi granules in the median of Golgi area (Golgi G./Med.) in suckling dams fed with a pelleted food and distilled water (Group I), with a 11.5% milk diet (Group II) and with a 11.5% milk diet supplemented with 1% urea (Group III) from day 13 to day 17 of lactation

	% r-ER	Secretory G./μm^2	Golgi G./μm^2	Golgi G./Med.[a]
Pellet.+D.W. (Group I)	11.9±1.0[b]	2.3±0.11	0.9±0.06	9.0 (3)
Milk diet (Group II)	17.9±1.0**	1.9±0.07*	1.2±0.09°	12.6 (6)
Milk+Urea (Group III)	36.9±1.1**,††	1.0±0.05**,††	1.5±0.09**,†	25.5 (6)

[a] Total number of Golgi granules in the median of the Golgi area was obtained multiplying the number of Golgi granules per unit area by the median of the Golgi area. [b] Mean±S.E.M., Group I vs. Group II and Group III; * $p<0.01$ ** $p<0.001$. Group II vs. Group III; † $p<0.01$ †† $p<0.001$. No. of animals is shown in parentheses.

with a planimeter (X-PLAN 360, Ushikata, Tokyo). The number of secretory granules and of Golgi immature granules was counted in each cell. The numerical density of secretory granules and of Golgi immature granules was calculated, in which the cytoplasmic area for secretory granules was estimated subtracting the Golgi area from the whole cytoplasm of the cell. The percent area of the cytoplasm occupied by the r-ER was obtained with a Planimex 25 (Nihon Regulator, Japan). Statistical significance was assessed with Student's t-test.

RESULTS

1. *Oral intake of distilled water and of liquid milk diet supplemented with or without urea*

During the experimental period of lactation from day 13 to day 17, suckling dams (Group I) drank distilled water in the mean of 7.5±1.5 ml/10 g b.w./day (Table 1). This water consumption of lactating mice was about 4 times as much as that of age matched unlliparous female mice (1.8±0.3 ml/10 g b.w./day, data not presented). When suckling mice were deprived of a pelleted food and, instead, given a 11.5% milk diet (Group II) their daily liquid consumption increased to 181%, 177%, 191%, 266% and 193% of the each corresponding control value (Group I), respectively, from day 13 to day 17 of lactation (Table 1). Likewise, lactating mice given a 11.5% milk diet plus 1% urea (Group III) showed copious drinking in a range of 171%, 179%, 221% 266% and 216% of the each control value (Group I) during the same period from day 13 to day 17 of lactlation (Table 1).

2. *Hematocrit and plasma osmolality*

Hematocrit of the blood increased to 118% in suckling mice given a liquid milk diet (Group II) and to 116% in those fed a milk plus urea diet (Group III) when compared to that of the control (Table 2). Plasma osmolality in the Group II and Group III was not significantly different from that of the control Group I (Table 2).

3. *Electron microscopy and morphometry of PI cells of the pituitary gland*

In the control suckling mice (Group I) PI cells of the pituitary appeared to contain numerous secretory granules in the cytoplasm. The rough endoplasmic reticulum (r-ER) was pooly developed (Fig. 1). Whereas PI cells of the suckling dams given milk plus urea liquid regimen showed a cellular sign of hypersecretion; well developed r-ER, extensive Golgi apparatus and sparse secretory granules in the cytoplasm (Fig. 2).

The percent area of the cytoplasm occupied by the r-ER in suckling mice given liquid milk diet (Group II) and in those fed milk plus urea diet (Group III) increased to 150% and 310% of the control value, respectively (Table 3). The numerical density of immature Golgi granules in the Group II and Group III increased to 133% and 167% of the control value, respectively. In this case, however, the Golgi apparatus showed marked hypertrophy especially in those of the Group III. Thus the theoretical number of Golgi granules per the median of Golgi area was culculated. The total Golgi granules of the median Golgi area increased to 140% and 283% of the control value in the Group II and Group III, respectively (Table 3). On the contrary, the numerical density of secretory granules in the cytoplasm decreased to 83% and to 43% of the control value in the Group II and Group III, respectively.

DISCUSSION

The present study demonstrated an enhanced activity in cells of the pars intermedia (PI) of the pituitary in suckling mice given either a 11.5% liquid milk diet or a 11.5% milk diet supplemented with 1% urea for 5 days from day 13 to day 17 of lactation. It is of interest to describe here that oral intake of a urea plus milk diet resulted in marked hypersecretion in PI cells of suckling dams more significantly than that of a milk diet only.

The development of polydipsia and polyurea in old male rats of the Wistar/Tw strain at the age of 16 months has been reported [16–18], but the studies have not focused on the cytology of pituitary PI cells. Polydipsia (137% v/w/day) in mice with hereditary nephrogenic diabetes inspidus (DI) associated with marked hypertrophic and hyperplastic PI has been well documented [12, 13, 19]. Further, electron microscopic observations have revealed that predominant light PI cells contain many electron dense core granules and the sparse endoplasmic reticulum [20]. Thus, polydipsia in DI mice exerts also little effects on the synthesis and release of hormones of PI cells in fine structure. Therefore, it seems reasonable to assume that these polydipsic animals including DI mice as well as normal suckling dams (ca. 75% v/w/day) are in a condition under which hydromineral metabolism is regulated within a physiological range. What is the exact meaning of a physiological range in polydipsia remains to be disclosed.

When the pelleted food is absent, suckling dams drink a large amount of liquid milk (164% v/w/day) during the test period, and their pituitary PI cells show small but significant hypersecretion in fine structure (Table 3). In this case animals are considered to be in a nutritionally balanced condition since they are fed with a 11.5% milk diet that is the same concentration used in a human baby. Thus this small change in the PI cell morphology despite copions drinking may be due to either a supply of minerals from a milk diet or less amount of water intake than that of the case of a 5% glucose regimen (oral intake ca. 200% v/w/day) [9].

An interesting new finding in the present study is unexpected hypersecretion in PI cells of suckling dams given a 11.5% milk plus 1% urea diet. Morphometric data in two dimensional analysis clearly indicate the sigificance of hypersecretion of PI cells. The percent area of the cytoplasm occupied by r-ER, a parameter of protein synthesis, is 2.06 times; and the number of Golgi immature granules, a capacity of granule formation, is 2.02 times; whereas the numerical density of secretory granules reflecting the release of secretory granules is 0.53 times, respectivety, when compared to those of suckling mice given a milk diet only (Table 3). These results imply that the rate of degranulation exceeded the rate of both increased protein synthesis and granule formation in PI cells by twice in magnitude in suckling dams fed a urea plus milk diet. This urea-induced activation of PI cells in mice during lactation is the first report in the present study.

An abnormally high blood urea concentration and increased serum osmolality are apparently associated with the hypertrophy (but not with the hypersecretion) of the pars intermedia of mice with hereditary nephrogenic diabetes inspidus [12, 24]. In their studies, however, causative relationships between the hypertrophy of the PI and the high blood urea concentration in DI mice are not explained. Although it is generally assumed that the cell membranes are freely permeable to urea this assumption has met the conflicting data [21–23]. A high blood urea nitrogen (BUN) concentration is associated with a shift of water from the cells into the extracellular water and blood. Therefore, when the blood urea nitrogen is high, there is variable lowering of the serum sodium concentration due to dilution of the extracellular water [23]. It is uncertain in the present study whether the same mechanism of lowering sodium concentration by a high blood urea nitrogen is involved. Alternatilvely, PI peptids(s) being released by oral intake of excessive urea plus milk diet may have some pivotal functions other than the stimulation of aldosterone secretion by the adrenals as reported before [4, 5]. Further studies on urea mediated activation of PI cells in mice are in progress.

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